U.S.S.N. 09/582,534
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AMENDMENT AND RESPONSE TO OFFICE ACTION

In the Claims

Claims 1-81 were previously canceled.

82. (once amended) A recombinant host cell comprising ~~a~~ nucleic acid ~~segment~~ segments encoding a non-naturally occurring fusion ~~protein~~ proteins, wherein ~~the~~ a first nucleic acid segment comprises:

a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

a nucleic acid sequence encoding a polyhydroxyalkanote synthase protein subunit; and

a second segment comprising:

a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

a nucleic acid segment encoding an enzyme selected from the group consisting of acyl-ACP thioesterase, fatty acyl hydroxylase, yeast multifunctional protein (MFP), and hydroxyacyl-CoA epimerase.

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83. (original) The recombinant host cell of claim 82, wherein the recombinant host cell is a fungal cell.

84. (original) The recombinant host cell of claim 83, wherein the fungal cell is a *Schizosaccharomyces pombe, Streptomyces rimofaciens, Fusarium, Aspergillus niger, or Saccharomyces cerevisiae* cell.

85. (original) The recombinant host cell of claim 82, wherein the recombinant host cell is a plant cell.

86. (twice amended) The recombinant host cell of claim 85, wherein the plant cell is selected from the group of cells obtained from a plant consisting of ~~the~~ alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, ~~or~~ and wheat ~~cell~~.

87. (original) The recombinant host cell of claim 82, further comprising a nucleic acid segment encoding an acyl-ACP thioesterase.

88. (original) The recombinant host cell of claim 82, further comprising a nucleic acid segment encoding a fatty acyl hydroxylase.

89. (original) The recombinant host cell of claim 82, further comprising a nucleic acid segment encoding a yeast multifunctional protein (MFP).

90. (original) The recombinant host cell of claim 82, further comprising a nucleic acid segment encoding a hydroxyacyl-CoA epimerase.

91. (once amended) A genetically transformed plant cell comprising in the 5' to 3' direction:

a) a promoter to direct transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:

i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;

b) a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:

i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;

c) a 3' transcription terminator sequence; ~~and~~

d) a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence; and

e) a promoter to direct transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises: a nucleic acid sequence encoding a peroxisome targeting protein subunit; and a nucleic acid segment encoding an enzyme selected from the group consisting of acyl-ACP thioesterase, fatty acyl hydroxylase, yeast multifunctional protein (MFP), and hydroxyacyl-CoA epimerase;

f) a 3' transcription terminator sequence; and

g) a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence.

92. (twice amended) The genetically transformed plant cell of claim 91, wherein the plant cell is obtained from a plant selected from the group consisting of ~~the~~ alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, ~~or~~ and wheat ~~cell~~.

93. (original) The genetically transformed plant cell of claim 91, further comprising a nucleic acid segment encoding an acyl-ACP thioesterase.

94. (original) The genetically transformed plant cell of claim 91, further comprising a nucleic acid segment encoding a fatty acyl hydroxylase.

95. (original) The genetically transformed plant cell of claim 91, further comprising a nucleic acid segment encoding a yeast multifunctional protein (MFP).

96. (original) The genetically transformed plant cell of claim 91, further comprising a nucleic acid segment encoding a hydroxyacyl-CoA epimerase.

97. (once amended) A genetically transformed plant comprising in the 5' to 3' direction:

a) a promoter to direct transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:

i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;

b) a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:

i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;

c) a 3' transcription terminator sequence; ~~and~~

d) a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence; and

e) a promoter to direct transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises: a nucleic acid sequence encoding a peroxisome targeting protein subunit; and a nucleic acid segment encoding an enzyme selected from the group consisting of acyl-ACP thioesterase, fatty acyl hydroxylase, yeast multifunctional protein (MFP), and hydroxyacyl-CoA epimerase;

f) a 3' transcription terminator sequence; and

g) a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence.

98. (once amended) The genetically transformed plant of claim 91, wherein the plant is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell.

99. (original) The genetically transformed plant of claim 97, wherein the promoter is constitutive.

100. (original) The genetically transformed plant of claim 99, wherein the promoter is CaMV35S, enhanced CaMV35S, FMV, mas, nos, or ocs.

101. (original) The genetically transformed plant of claim 97, wherein the promoter is inducible.

102. (original) The genetically transformed plant of claim 101, wherein the promoter is tac, salicyclic acid induced, polyacrylic acid induced, safener induced, heat shock promoter, nitrate induced, hormone induced, or light induced.

103. (original) The genetically transformed plant of claim 97, wherein the promoter is tissue specific.

104. (original) The genetically transformed plant of claim 103, wherein the promoter is the β-conglycinin 7S promoter, napin promoter, phaseolin promoter, zein promoter, soybean trypsin inhibitor promoter, ACP promoter, stearoyl-ACP desaturase promoter, or oleosin promoter.

105. (original) The genetically transformed plant of claim 97, further comprising a nucleic acid segment encoding an acyl-ACP thioesterase.

106. (original) The genetically transformed plant of claim 97, further comprising a nucleic acid segment encoding a fatty acyl hydroxylase.

107. (original) The genetically transformed plant of claim 97, further comprising a nucleic acid segment encoding a yeast multifunctional protein (MFP).

108. (original) The genetically transformed plant of claim 97, further comprising a nucleic acid segment encoding a hydroxyacyl-CoA epimerase.

109. (twice amended) A method of preparing host cells useful to produce a non-naturally occurring fusion protein comprising the steps of:

a) selecting a host cell;

b) transforming the selected host cell with a first recombinant vector having a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid comprises:

i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit; and

c) transforming the selected host cell with a second recombinant vector having a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid comprises:

a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

a nucleic acid segment encoding an enzyme selected from the group consisting of acyl-ACP thioesterase, fatty acyl hydroxylase, yeast multifunctional protein (MFP), and hydroxyacyl-CoA epimerase; and

~~c)~~ d) obtaining transformed cells.

110. (original) The method of claim 109, wherein the vector further comprises a selectable marker.

111. (presently amended) The method of claim 110, wherein the selectable marker is selected from the group consisting of a kanamycin resistance marker, a hygromycin resistance marker, and ~~or~~ herbicide resistance marker.

112. (original) The method of claim 109, wherein the host cell is a fungal cell.

113. (original) The method of claim 112, wherein the fungal cell is a *Schizosaccharomyces pombe, Streptomyces rimofaciens, Fusarium, Aspergillus niger, or Saccharomyces cerevisiae* cell.

114. (original) The method of claim 109, wherein the host cell is a plant cell.

115. (twice amended) The method of claim 114, wherein the plant cell from a plant selected from the group consisting of ~~of~~ alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, and ~~of~~ wheat.

116. (twice amended) A method of preparing a transformed plant useful to produce a non-naturally occurring fusion protein comprising the steps of:

a) selecting a host cell;

b) transforming the selected host cell with a first recombinant vector having a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:

i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and

ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;

c) transforming the selected host cell with a second recombinant vector having a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:

i) a nucleic acid sequence encoding a peroxisome targeting protein subunit and

ii) a nucleic acid segment encoding an enzyme selected from the group consisting of acyl-ACP thioesterase, fatty acyl hydroxylase, yeast multifunctional protein (MFP), and hydroxyacyl-CoA epimerase; and

~~c)~~ d) obtaining transformed host plant cells; and

~~d)~~ e) regenerating the transformed host plant cells.

117. (original) The method of claim 116, wherein the vector further comprises a selectable marker.

118. (original) The method of claim 117, wherein the selectable marker is a kanamycin resistance marker, a hygromycin resistance marker, or a herbicide resistance marker.

119. (once amended) The method of claim 116, wherein the host plant cell is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell.

120. (original) The plant produced by the method of claim 116.

121. (once amended) A method for the preparation of a polyhydroxyalkanoate, comprising the steps of:

a) obtaining a cell capable of producing a non-naturally occurring first fusion protein, wherein the fusion protein comprises:

i) a peroxisome targeting protein subunit; and

ii) a polyhydroxyalkanoate synthase protein subunit; and

wherein the cell is capable of producing a second non-naturally occurring fusion protein, wherein the fusion protein comprises:

i) a peroxisome targeting protein subunit; and

ii) an enzyme selected from the group consisting of acyl-ACP thioesterase, fatty acyl hydroxylase, yeast multifunctional protein (MFP), and hydroxyacyl-CoA epimerase; and

b) establishing a culture of the cell; and

c) culturing the cell under conditions suitable for the production of the polyhydroxyalkanotate.

122. (original) The method of claim 121, wherein the culture contains natural fatty acids, non-natural fatty acids, or mixtures thereof.

123. (original) The method of claim 121, wherein the cell is a fungal cell.

124. (original) The method of claim 123, wherein the fungal cell is a *Schizosaccharomyces pombe, Streptomyces rimofaciens, Fusarium, Aspergillus niger, or Saccharomyces cerevisiae* cell.

125. (original) The method of claim 121, wherein the cell is a plant cell.

126. (twice amended) The method of claim 125, wherein the cell is from a plant selected from the group consisting of ~~an~~ alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive,

palm, parsnip, pea, peanut, pepper, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, ~~or~~ and wheat cell.

127. (original) The method of claim 121, wherein the polyhydroxyalkanoate comprises 3-hydroxyhexanoic acid (H:6), 3-hydroxyoctanoic acid (H:8), 3-hydroxydecanoic acid (H:10), 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14), 3-hydroxyhexadecanoic acid (H:16), 3-hydroxyheptanoic acid (H:7), 3-hydroxynonanoic acid (H:9), 3-hydroxyundecanoic acid (H:11); 3-hydroxytridecanoic acid (H:13), 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2), 3-hydroxydodecenoic acid (H12:1), 3-hydroxyoctenoic acid (H8:1), 4-hydroxydecanoic acid, 8-methyl-3-hydroxynonanoic acid, or 6-methyl-3-hydroxyheptanoic acid monomers.

128. (once amended) A method for the preparation of a polyhydroxyalkanoate, comprising the steps of:

a) obtaining a plant capable of producing a non-naturally occurring first fusion protein, wherein the fusion protein comprises:

i) a peroxisome targeting protein subunit; and

ii) a polyhydroxyalkanoate synthase protein subunit; and wherein the plant is capable of producing a second non-naturally occurring fusion protein, wherein the fusion protein comprises:

i) a peroxisome targeting protein subunit; and

ii) an enzyme selected from the group consisting of acyl-ACP thioesterase, fatty acyl hydroxylase, yeast multifunctional protein (MFP), and hydroxyacyl-CoA epimerase; and

b) growing the plant under conditions suitable for the production of the polyhydroxyalkanoate.

129. (original) The method of claim 128, further comprising supplementing the plant with natural fatty acids, non-natural fatty acids, or mixtures thereof.

130. (once amended) The method of claim 128, wherein the plant is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, [potato,] radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat plant.

131. (original) The method of claim 128, wherein the polyhydroxyalkanoate comprises 3-hydroxyhexanoic acid (H:6), 3-hydroxyoctanoic acid (H:8), 3-hydroxydecanoic acid (H:10), 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14), 3-hydroxyhexadecanoic acid (H:16), 3-hydroxyheptanoic acid (H:7), 3-hydroxynonanoic acid (H:9), 3-hydroxyundecanoic acid (H:11); 3-hydroxytridecanoic acid (H:13), 3-

hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2), 3-hydroxydodecenoic acid (H12:1), 3-hydroxyoctenoic acid (H8:1), 4-hydroxydecanoic acid, 8-methyl-3-hydroxynonanoic acid, or 6-methyl-3-hydroxyheptanoic acid monomers.

132. (original) A plant containing a polyhydroxyalkanoate, wherein the polyhydroxyalkanoate comprises 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14), 3-hydroxyhexadecanoic acid (H:16), 3-hydroxynonanoic acid (H:9), 3-hydroxyundecanoic acid (H:11); 3-hydroxytridecanoic acid (H:13), 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2), 3-hydroxydodecenoic acid (H12:1), 3-hydroxyoctenoic acid (H8:1), 4-hydroxydecanoic acid, 8-methyl-3-hydroxynonanoic acid, or 6-methyl-3-hydroxyheptanoic acid monomers.

133. (please cancel) A polyhydroxyalkanoate comprising 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxydodecadienoic acid (H12:2) monomers.